

Amendments to the Specification:

- Please replace the paragraph bridging pages 8 and 9 with the following text:

Figures 11A-11D show that rictor is a novel mTOR-associated protein. (A) Silver stain of SDS-PAGE analysis of mTOR immunoprecipitates prepared from HeLa cells lysed in a CHAPS- or Triton X-100-containing buffer. (+) indicates inclusion of the blocking peptide for the mTOR antibody during the immunoprecipitation. The ~200 kDa band corresponds to rictor and a non-specific band (NS) obscures raptor. (B) Rictor homologues share common domain architectures. Analyses of indicated rictor homologues identified seven domains with sequence conservation and similar relative locations within each protein and are shown schematically as boxes. Domain five is repeated four times within each of the homologues and the multiple sequence alignment shows the sequence pattern of this repeat. The amino acid sequences shown on line 1 to line 20 are designed as SEQ ID NOs: 29-48, respectively. Sequences with the following accession numbers were used to create the alignment: D. melanogaster, AAQ22398.1; A. gambiae, XP_309233.1; H. sapiens, AY515854; D. discoideum, AAC35553.1; S. pombe, NP_596021.1; S. cerevisiae, NP_011018.1. (C) Specific interaction between endogenous mTOR and rictor. Immunoprecipitates prepared with the indicated antibodies were analyzed by immunoblotting for mTOR, rictor and raptor. Prior to use cells were treated with 5 μ M Antimycin A for 15 min (Antimy), 20 nM rapamycin for 15 min (Rapa), deprived of leucine for 90 min (-Leu), or deprived of leucine and stimulated with 52 μ g/ml leucine for 10 min (-Leu+Leu). (D) Endogenous mTOR interacts with recombinant rictor and raptor. Cellular lysates and mTOR immunoprecipitates prepared from HEK293T cells expressing myc-rictor, myc-raptor, or myc-GCP3 were analyzed by immunoblotting for myc-tagged proteins. In parallel, anti-myc immunoprecipitates were analyzed by immunoblotting for mTOR.

- On page 11, please replace the paragraph starting at line 3 with the following text:

Figure 17 shows the amino acid sequence of the G β L protein and the seven WD40 repeats in this protein. The amino acid sequence shown is designed as SEQ ID NO: 49.

- On page 55, please replace the paragraph starting at line 8 with the following text:

Reagents were obtained from the following sources: protein G-sepharose from Pierce; ATP- $[\gamma\text{-}^{32}\text{P}]$ from NEN; mTOR, S6K1, and PKC α antibodies as well as HRP-labeled anti-mouse, anti-goat, and anti-rabbit secondary antibodies from Santa Cruz Biotechnology; phospho-T389 S6K1 and phospho-PKC α/β_2 antibodies from Cell Signaling; HA monoclonal antibody from Covance; myc monoclonal antibody from Oncogene Research Products; Drosophila S6K antibody from Mary Stewart, North Dakota State University; Alexa Fluor 488-conjugated secondary anti-mouse antibody and Texas Red-X-phalloidin from Molecular Probes; paxillin monoclonal antibody from BD Transduction Laboratories; DMEM, leucine, glucose, RPMI, and RPMI without leucine from Life Technologies; rapamycin, LY294002, and antimycin A from Calbiochem. The G β L antibody was described previously (Kim et al., 2002, Cell, 110:163-175), and the rictor and raptor antibodies were developed with the antibody service from Covance using the following peptides: (rictor: RGRSLKNLRVRGRND, amino acid sequence 6-20, designed as SEQ ID NO: 50) and (raptor: mesemlqspllglgeedead, amino acid sequence 1-20, designed as SEQ ID NO: 51).

➤ Please replace the paragraph bridging pages 57 and 58 with the following text:

Effectene (Qiagen) was used to transfect 1.2 million HEK293T cells in 6-cm dishes with up to 1 μg of the expression plasmids indicated in the figure legends. 48 hours after DNA addition, the cells were rinsed once with PBS and lysed in 800 μl of ice-cold Lysis Buffer containing either CHAPS or Triton X-100 and analyzed by immunoprecipitation and immunoblotting as above. Sequences and transfection conditions for synthetic siRNAs targeting lamin, mTOR, and raptor have been described (Kim et al., 2002, Cell, 110:163-175) and are available at http://web.wi.mit.edu/sabatini/pub/siRNA_sequences.html. The sequences of the sense and anti-sense strands of the siRNA targeting rictor are ACUUGUGAAGAAUCGUAUCdTdT (SEQ ID NO: 7) and dTdTUGAACACUUCUAGCAUAG (SEQ ID NO: 8), respectively. Those for PKC α are UCCUUGUCCAAGGAGGCUGdTdT (SEQ ID NO: 9) and dTdTAGGAACAGGUUCCUCCGAC (SEQ ID NO: 10).

- On page 58, please replace the paragraph starting at line 14 with the following text:

The sequences of the oligonucleotides are as follows:

(1) mTOR 609 sense (SEQ ID NO: 11):

CCGGTTCAGCGTCCCTACCTTCTTCTctcgagAGAAGAAGGTAGGGACGCTGATTTTGTG.

(2) mTOR 609 antisense (SEQ ID NO: 12):

AATTCAAAAATCAGCGTCCCTACCTTCTTCTctcgagAGAAGAAGGTAGGGACGCTGA
A.

(3) Raptor 4145 sense (SEQ ID NO: 13):

CCGGagggccctgctactcgttttctcgagaaaagcgagtagcagggccctTTTTTG.

(4) Raptor 4145 antisense (SEQ ID NO: 14):

AATTCAAAAaagggccctgctactcgttttctcgagaaaagcgagtagcagggccct.

(5) Rictor 3274 sense (SEQ ID NO: 15):

CCGGTACTTGTGAAGAATCGTATCTTctcgagAAGATACGATTCTTCACAAGTTTTTTTG.

(6) Rictor 3274 antisense (SEQ ID NO: 16):

AATTCAAAAACCTTGTGAAGAATCGTATCTTctcgagAAGATACGATTCTTCACAAGTA

- On page 59, please replace the paragraph starting at line 17 with the following text:

(1) EGFP forward (SEQ ID NO: 17): ATGGTGAGCAAGGGCGAGGAGCTGT;

(2) EGFP reverse (SEQ ID NO: 18): TTAATTGTACAGCTCGTCCATGCCG;

(3) dTOR (CG5092) forward (SEQ ID NO: 19): CAGGAGTTATTTTAAATGTGCTTCG;

(4) dTOR reverse (SEQ ID NO: 20): CCAAAATTCTTTGATCAGCTTAAAA;

(5) dRaptor (CG4320) forward (SEQ ID NO: 21): TGTCTGACAACACCCATTAACATAG;

(6) dRaptor reverse (SEQ ID NO: 22): GTACTTGTATTCCTTGACCAGATCC;

(7) dRictor (CG8002) forward (SEQ ID NO: 23): GCTTATTCCTAGACAGCATTATCCA;

(8) dRictor reverse (SEQ ID NO: 24): TTTTGAGTACTTCGATGCCTTTTAC;

(9) dS6K (CG10539) forward (SEQ ID NO: 25): CCTTCATAGTGGAGCTAGTTTATGC;

(10) dS6K reverse (SEQ ID NO: 26): CTTAGCGTTGTATCATCAGGTGAAT.

- please replace the paragraph bridging pages 59 and 60 with the following text:

Each primer included a GAA and T7 promoter sequence (GAATTAATACGAC TCACTATAGGGAGA, SEQ ID NO: 27) at its 5' end. Primers were used in a one-step RT PCR reaction (Qiagen) to amplify a cDNA fragment using total *Drosophila* S2 cell RNA as template. The total RT-PCR reaction was purified using a PCR purification column (Qiagen) in a final volume of 40 µl. 8 µl of the RT-PCR product was then used as a template in a 20 µl in vitro transcription reaction using the Megascript kit (Ambion) to generate the corresponding dsRNA fragments. The GFP template was amplified from an EGFP expression plasmid (Stratagene). *Drosophila* S2 cells actively growing in Schneider medium (Life Technologies) were washed and resuspended in *Drosophila* SFM (Life Technologies) to a final density of 1×10^6 cells in 1 ml volume. 30 µg of dsRNA was added to the 1 ml of cells in SFM and incubated for 45 min at 25 °C. 2 ml of Schneider medium with 10% serum was then added back to the cells. After 24 hours, the cells were starved again and an additional 30 µg of dsRNA was added. After 4 days cells were harvested, washed once with cold PBS, lysed in the 1% Triton X-100 Lysis Buffer and analyzed by immunoblotting as above. Antibodies developed against mammalian phospho-S6K1, phospho-PKCα and PKCα were used to detect the *Drosophila* homologues of these proteins/modifications.